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Review article

Maternal monocytes in pregnancy and preeclampsia in humans and in rats

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ABSTRACT

Monocytes are short-lived cells, arising from the bone marrow and maturing in the circulation. They play an important role in immune responses and are thought to be important for healthy pregnancy. In humans, 3 subpopulations of monocytes have been identified: classical, intermediate and non-classical monocytes. These subpopulations have different functions and phenotypical characteristics. Healthy pregnancy is characterized by a pro-inflammatory condition, with increased numbers of monocytes and monocyte activation as well as with increased numbers of intermediate monocytes and decreased numbers of classical monocytes. This may suggest monocyte maturation. Preeclampsia is an important pregnancy complication characterized by hypertension and proteinuria developing in the second half of pregnancy. The pathophysiology of preeclampsia is associated with further activation of the inflammatory response, further activation of monocytes and further monocyte maturation. In the present review we focus on the role of monocyte activation and maturation in healthy and preeclamptic pregnancy.

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1. Introduction

Pregnancy poses a unique immunological challenge to the mother. Semi-allogeneic placental tissue is in direct contact with circulating and uterine maternal immune cells. Therefore adaptations in the immune response are seen locally in the uterus and decidua, but also peripherally in the maternal blood (Veenstra Van Nieuwenhoven et al., 2003b). It has been suggested that the adaptations of the peripheral immune response are due to the circulation of maternal blood through the placenta and the secretion of placental factors into the maternal circulation (Sacks et al., 1999; Mellembakken et al., 2002). Adaptations in the maternal peripheral immune response are observed in the specific immune response, such as a decreased Th1/Th2 ratio in T cells (Wegmann et al., 1993; Saito et al., 1999; Veenstra Van Nieuwenhoven et al., 2002), increased numbers of regulatory T cells during the first and second trimester of pregnancy (Saito et al., 2010; Ernerudh et al., 2011) and an increased Treg/Th17 ratio (Figueiredo and Schumacher, 2016). Changes are also observed in NK cells (Veenstra Van Nieuwenhoven

et al., 2002; Borzychowski et al., 2005). Not only cells of the adaptive immune response, but also cells of the innate immune system, monocytes and granulocytes, are affected by pregnancy. They show an activated phenotype (Sacks et al., 1998).

Preeclampsia is a major complication of the second half of pregnancy. It is characterized by hypertension and proteinuria (Duley, 2009; Steegers et al., 2010). The most severe form of preeclampsia, early onset preeclampsia, is thought to arise from poor placentation (Redman and Sargent, 2009). This results in the production of pro-inflammatory factors by the diseased placenta into the maternal circulation (Hung et al., 2004; Levine et al., 2004; Germain et al., 2007; Spaans et al., 2014a). Such factors may further activate the already activated monocytes in pregnancy and together with activation of other inflammatory cells, such as granulocytes and endothelial cells, finally induce the full blown syndrome of preeclampsia (Redman and Sargent, 2009).

The present review will focus on peripheral monocytes in pregnancy and preeclampsia. Changes in circulating monocytes will be discussed as well as the role these cells may play in the physiology of normal pregnancy and the pathophysiology of preeclampsia. Animal models will be discussed since they are important in understanding the role of monocytes pregnancy and preeclampsia.

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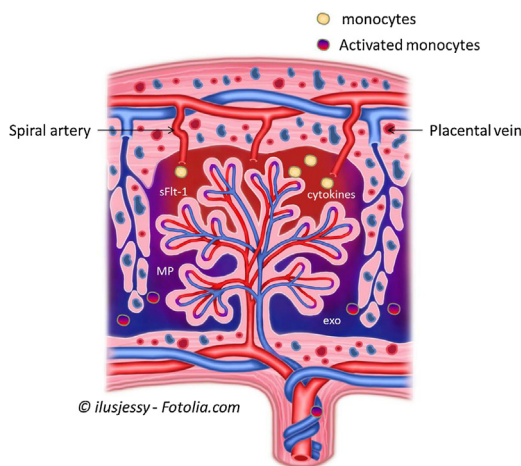


Fig. 1. Monocytes in the utero-placental circulation.

Monocytes get into the intervillous space via the spiral arteries. In the intervillous space they can come into direct contact with the fetal syncytiotrophoblast. The syncytiotrophoblast also produces various factors into the intervillous space (and thus into the maternal circulation), such as syncytiotrophoblast microparticles (MP) or exosomes (exo), cytokines, antiangiogenic factors (such as sFlt-1). Such factors may also activate monocytes in the intervillous space. Activated monocytes leave the intervillous space via the placental veins to get into the maternal circulation.

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1.1. Utero-placental circulation

The placenta is important in maternal-fetal exchange of nutrients and gases. In a human placenta, which is hemochorial, this is enabled by fetal villi bathing in maternal blood in the intervillous space (Boyd JD, 2013). The placenta develops after invasion of fetal trophoblast into the endometrium and the spiral arteries in the endometrium starting 5–6 days after fertilization (Sadler, 2004). Although, the development of the villous structure and the intervillous space starts early in pregnancy, the connection of the intervillous space with the spiral arteries does only develop after 9 weeks of pregnancy (Jauniaux et al., 2000). From that time onward, maternal blood is in close contact with the fetal villi. This enables easy exchange of gases and nutrients between mother and fetus, but also poses a challenge to the mother, since maternal immune cells circulating through the placenta in the intervillous space are in direct contact with fetal semi-allogeneic trophoblast cells. This contact of maternal immune cells with the fetal trophoblast may influence the maternal immune cells. Not only direct contact between fetal trophoblast and maternal immune cells may influence the maternal immune cells, it has also been suggested that the trophoblast secretes many factors into the maternal circulation that also may affect the maternal immune cells. Such factors may be cytokines (Sacks et al., 2001), fetal DNA (Bianchi et al., 1996) and syncytiotrophoblast microvesicles (Redman et al., 2012) (see Fig. 1).

1.2. Monocytes

Monocytes are short-lived circulating cells, which arise from myelo-monocytic precursors in the bone marrow. They comprise about 5–10% of the circulating blood leukocytes and migrate into the tissue to become macrophages and dendritic cells (Gordon and Taylor, 2005). They have various functions, such as phagocytosis, antigen presentation and cytokine production (Gordon and Taylor, 2005). Monocytes in the peripheral circulation have a heterogeneous morphology, since they can vary in size, show different degrees of granularity and may have different nuclear morphology

(Gordon and Taylor, 2005). In humans, monocytes can be identified by expression of the extracellular marker CD14, which is expressed by all monocytes (Gordon and Taylor, 2005).

1.2.1. Monocyte subsets

In humans, three monocyte subsets exist, which can be distinguished based on their expression of CD14 and CD16 (FcγR-III). The main subset, the classical monocytes, representing 90–95% of the monocytes, is a subset, which expresses high levels of CD14, while lacking CD16 expression. Non-classical monocytes are the second subset, which is characterized by low expression of CD14 with high expression of CD16. A third, intermediate subset of monocytes (high CD14 expression and high CD16 expression) has also been defined (Ziegler-Heitbrock et al., 2010). It is thought that classical monocytes arise from the bone marrow and mature in the circulation via intermediate monocytes into non-classical monocytes (Sunderkötter et al., 2004; Ziegler-Heitbrock et al., 2010). The monocyte subsets differ in many aspects (Gordon and Taylor, 2005; Ziegler-Heitbrock et al., 2010). Classical monocytes are able to generate reactive oxygen species (ROS) and produce cytokines after stimulation with Toll-like receptor agonists. They are strong phagocytes. Non-classical monocytes do not generate ROS, but are more efficient producers of pro-inflammatory cytokines after TLR stimulation, while they are weaker phagocytes (Gordon and Taylor, 2005). The non-classical monocytes have a longer half-life and infiltrate into resting and inflamed tissue (Gordon and Taylor, 2005). They are thought to patrol and survey the endothelium and rapidly invade the tissue and initiate the inflammatory response (Auffray et al., 2007; Ziegler-Heitbrock et al., 2010). The function of intermediate monocytes is less clear and in general they have an intermediate function between classical and non-classical monocytes (Wong et al., 2011). Based on their high gene and protein expression of MHC II (Wong et al., 2011; Groen et al., 2015), they may have an important role in antigen presentation and T cell activation. Intermediate monocytes have been shown to be increased in various inflammatory diseases (Rogacev et al., 2012; Ziegler-Heitbrock, 2015; Wong et al., 2012), suggesting that they play a pathophysiological role in these diseases.

2. Maternal monocytes in pregnancy

Changes in the innate immune response are apparent during pregnancy. Most obvious are the increased numbers of circulating monocytes and granulocytes (Siegel and Gleicher, 1981; Kuhnert et al., 1998; Veenstra Van Nieuwenhoven et al., 2003a). However, not only are numbers of monocytes increased during pregnancy, also the monocytes are activated and show functional changes. Phenotypical activation of monocytes during pregnancy has been shown by increased expression of the activation markers CD11b, CD14 and CD64 on monocytes from pregnant women as compared with monocytes from non-pregnant women (Sacks et al., 1998; Naccasha et al., 2001; Luppi et al., 2002a,b). Functional changes in monocytes from pregnant women have been demonstrated by increased production of oxygen free radicals (Sacks et al., 1998) and decreased phagocytosis in pregnancy (Lampe et al., 2015). Also changes in cytokine production have been observed (Veenstra Van Nieuwenhoven et al., 2003a; Faas et al., 2014a). Cytokine production can be studied in unstimulated and stimulated monocytes. Unstimulated monocyte cytokine production represents the *in vivo* cytokine production, while stimulated monocyte cytokine production represents the ability of monocytes to respond to stimuli *in vivo*. For unstimulated monocytes, results have been inconsistent, since increased (Luppi et al., 2002a), decreased (Faas et al., 2014a) or unchanged (Veenstra Van Nieuwenhoven et al., 2003a;

Germain et al., 2007) cytokine production by non-stimulated monocytes from pregnant women vs non-pregnant women has been shown. These inconsistent results may be due to different methods of preparing monocytes and/or measuring cytokine production. Monocyte cytokine production can be measured in whole blood (Luppi et al., 2002a; Veenstra Van Nieuwenhoven et al., 2003a; Faas et al., 2014a), after isolating peripheral blood mononuclear cells (PBMC) (Germain et al., 2007), or after isolating monocytes (this has never been done for pregnant women). Isolation of monocytes, even a relatively mild isolation such as isolation of PBMC, may affect monocyte activation and therefore cytokine production (Macey et al., 1995). Also the method of measuring cytokine production may influence the outcome. Cytokines can be measured by ELISA (Germain et al., 2007; Faas et al., 2014a) or by flow cytometry (Luppi et al., 2002a; Veenstra Van Nieuwenhoven et al., 2003a). Especially for flow cytometry, the choice of the antibodies, dilution of the antibodies, and choice of the isotype controls as well as gate setting is extremely important and differences between different labs may result in different outcomes. Changes in unstimulated monocyte cytokine production in vitro may be in line with the fact that plasma cytokine levels have changed during pregnancy (Szarka et al., 2010). It has for instance been shown that plasma levels of IL-12 and IL-18 are increased during pregnancy (Szarka et al., 2010).

In order to study the potential of monocytes to respond to pro-inflammatory stimuli, in various studies monocytes have been stimulated. Often lipopolysaccharide (LPS) has been used. After stimulation with LPS, cytokine production by monocytes from pregnant women was decreased as compared with cytokine production by monocytes from non-pregnant women (Veenstra Van Nieuwenhoven et al., 2003a; Beckmann et al., 2004; Faas et al., 2014a). This decreased cytokine production is not due to decreased expression of TLR4 (which is the main receptor for LPS) (Faas et al., 2014a). It may, however, be a sign of activation of monocytes, since activated monocytes become tolerant to LPS (Faas et al., 2002). IFN γ is able to abrogate LPS tolerance (Chen and Ivashkiv 2010) and therefore, after stimulation of monocytes with both LPS and IFN γ , endotoxin tolerance can be overcome and monocytes of pregnant women showed increased cytokine production as compared with monocytes from non-pregnant women after stimulation with both LPS and IFN γ (Sacks et al., 2003). Most of the studies on monocytes during pregnancy have been performed in the third trimester of pregnancy and based on all above-mentioned data, it is now generally accepted that monocytes are activated during pregnancy. Unfortunately, not many studies have evaluated monocyte activation during the course of pregnancy. One study showed progressive phenotypical activation of monocytes from the first trimester to the third trimester (Luppi et al., 2002a).

2.1. Monocyte subsets in pregnancy

Although it has been known for more than a decade that monocytes are a heterogeneous population, studies on monocytes in pregnancy as presented above have mainly been performed on classical monocytes. Since intermediate monocytes are increased in inflammatory diseases, we hypothesized that this monocyte subset would also be increased in pregnancy. We thus conducted two studies in which we identified the 3 subsets of monocytes in pregnant women (Melgert et al., 2012; Groen et al., 2015). In line with our hypothesis, during healthy pregnancy we showed an increased number of intermediate monocytes and a decreased number of classical monocytes (Melgert et al., 2012; Groen et al., 2015). Al-ofi (Al-Ofi et al., 2012), however, showed different results, i.e. they showed a decreased number of non-classical monocytes in pregnant vs non-pregnant women and increased numbers of classical monocytes in pregnant women, with no

difference in intermediate monocytes. The reason for the different outcomes between these studies may be due to differences in flow cytometry methods, since in our studies (Melgert et al., 2012; Groen et al., 2015) we used whole blood for flow cytometry, while Al-ofi isolated PBMC (Al-Ofi et al., 2012). As indicated before, this latter method may activate monocytes and thus potentially change the ratio of monocyte subsets. The use of different antibodies and different labels may also explain differences in outcome, while also gating strategy may also have influenced outcome.

2.2. Monocytes in rodent pregnancy

Rodents, like humans have a hemochorial placenta, indicating that in both species maternal immune cells are in direct contact with fetal trophoblast. The rodent placenta, however, is not identical to the human fetal placenta, since the rodent placenta has a labyrinthine structure (Furukawa et al., 2014), rather than a villous structure. Also the rodent placenta is hemotrichorial rather than hemomonochorial as in humans (Furukawa et al., 2014). Moreover, the trophoblast facing the maternal blood space is cytotrophoblast in rodents, rather than syncytiotrophoblast as in humans (Furukawa et al., 2014). Rodents may thus be useful models to study the (patho) physiological role of maternal peripheral immune changes during pregnancy. Indeed, similar phenotypical and functional activation of monocytes during pregnancy have been observed in rats as compared with humans (Faas et al., 2000, 2004). These rat studies also showed that monocyte activation gradually developed during pregnancy (Faas et al., 2000, 2004). Unfortunately, although many studies have been done on immune responses in pregnant mice, nothing is known about monocyte activation in mouse pregnancy.

In rodents, like in humans, classical and non-classical monocytes have been described (Ziegler-Heitbrock, 2015). Although the functions of the subtypes are similar between humans and rodents, rodent monocyte subtypes are not identified by CD14 and CD16 expression but by other markers: for rats, we use CD172a and CD43 (Melgert et al., 2012), while for mice we use Ly6C (Elderman et al., 2016). In rats, usually only 2 types of subsets are identified, i.e. classical monocytes and non-classical monocytes (Melgert et al., 2012; Ziegler-Heitbrock, 2015). Since intermediate monocytes and non-classical monocytes have a relative similar gene profile (Wong et al., 2011), this may suggest that the distinction between non-classical and intermediate monocytes cannot be made in rats. In rats, we found decreased numbers of classical monocytes and increased numbers of non-classical monocytes during pregnancy (Melgert et al., 2012; Groen et al., 2013). In line with the above-mentioned data that the pro-inflammatory condition in rat pregnancy develops gradually, we found that the numbers of non-classical monocytes were increased as of day 13 of pregnancy (Melgert et al., 2012; Groen et al., 2013). In mice, similar to humans, 3 subsets of monocytes can be identified. We recently showed that in syngeneic pregnant mice (both BALB/c and B6 mice) numbers of intermediate monocytes are not increased as compared to non-pregnant mice (Elderman et al., unpublished observations). Since both humans and our rat models carry allogeneic fetuses, this may suggest that allogeneity is important for increasing the numbers of intermediate monocytes.

2.3. How is monocyte activation and maturation induced during pregnancy?

The exact mechanisms by which pregnancy induces monocyte activation and maturation are unknown. The placenta, however, seems to play an important role (see Figs. 1 and 2 a). As periph-

eral monocytes circulate through the placenta, they may come into direct contact with fetal syncytiotrophoblast, which may activate the monocytes. Indeed, *in vitro* studies have shown that monocytes can adhere to syncytiotrophoblast cells (Xiao et al., 1997). As many endothelial adhesion molecules are expressed on the syncytiotrophoblast (Dye et al., 2001), it seems likely that these adhesion molecules are involved in the adherences of monocytes to the syncytiotrophoblast. Indeed, adherence of monocytes to the syncytiotrophoblast has been shown to be mediated by ICAM-1-LFA-1 interactions (Xiao et al., 1997) or by fractaline (Siwetz et al., 2015). Such interactions may activate the monocytes, since it may induce the production of various pro-inflammatory cytokines (Butoi et al., 2011).

The fact that innate immune cells become activated after passage through the placental circulation (Mellembakken et al., 2002) may indeed suggest direct contact with the syncytiotrophoblast. However, it has also been shown that the syncytiotrophoblast secretes various factors into the maternal circulation, such as cytokines (Sacks et al., 2001), fetal DNA (Bianchi et al., 1996). *In vitro* studies have indeed shown that factors present in the plasma of pregnant women activate monocytes (Faas et al., 2010b). Also placental microparticles and exosomes are shed from the syncytiotrophoblast and may activate the monocytes (Germain et al., 2007; Redman et al., 2012; Gohner et al., 2015; Mitchell et al., 2015). Future studies are necessary to evaluate the relative importance of direct and indirect monocyte-syncytiotrophoblast contact on monocyte activation. Recently, it was shown that microparticles and exosomes not only activate monocytes but also induced maturation of monocytes from classical monocytes towards intermediate monocytes (Gohner et al., 2015). This may suggest that these extracellular particles play a role in the monocyte activation and maturation during pregnancy. Not only particles shed from the placenta, but also particles shed from other cells may activate monocytes (Sokolov et al., 2016).

3. Monocytes in preeclampsia

Preeclampsia is a serious complication of pregnancy, characterized by hypertension and proteinuria developing in the second half of pregnancy (Steegers et al., 2010). The first stage of preeclampsia is thought to be poor placentation or syncytiotrophoblast stress (Redman and Staff, 2015). The second stage is the production of pro-inflammatory factors from the diseased placenta into the maternal circulation, which further activate the inflammatory response, including monocytes and endothelial cells, finally resulting in the signs of preeclampsia (Redman and Sargent, 2009).

It has now been well established that during preeclampsia, the innate immune system is even further activated as compared with normal pregnancy (Borzychowski et al., 2006). Activation of monocytes in preeclampsia has been demonstrated by increased expression of activation markers, such as CD11b, ICAM-1, CD14 and TLR4 (Sacks et al., 1998; Gervasi et al., 2001; Mellembakken et al., 2002; Luppi et al., 2006; Chen et al., 2015) as compared with healthy pregnancy. However, monocytes are not only phenotypically activated, they also produce increased amounts of oxygen free radicals as compared to normal pregnancy (Sacks et al., 1998) and their cytokine production also differed as compared to monocytes from normal pregnant women: The difference, however, may depend on the method of measuring cytokine production, as IL-12 and TNF α were found to be increased (Sakai et al., 2002; Peraçoli et al., 2007; Brewster et al., 2008) or decreased (Veenstra Van Nieuwenhoven et al., 2008) in preeclampsia depending on the method used. The first papers measured LPS stimulated cytokine production using ELISA, while Veenstra van Nieuwen-

hoven measured LPS stimulated cytokine production using flow cytometry.

3.1. Monocyte subsets in preeclampsia

As for normal pregnancy, the above mentioned studies did not take into account the presence of monocyte subsets and thus monocytes in the above mentioned studies are generally only defined using CD14. Recently, it was shown that numbers of intermediate monocytes were even further increased and classical monocytes further decreased in preeclampsia as compared with healthy pregnancy (Melgert et al., 2012). Although these data were in line with data from Tang (Tang et al., 2015), Al-ofi showed increased numbers of non-classical monocytes in preeclamptic women compared with healthy pregnant women (Al-Ofi et al., 2012). As explained above, this may be due to different techniques used, but may also be due to a different selection of patient groups (inclusion of only early onset preeclamptic women, vs inclusion of a more heterogeneous group of preeclamptic women). Interestingly, Tang et al. showed that numbers of intermediate monocytes correlated with the severity of preeclampsia (Tang et al., 2015), suggesting that this monocyte subset may play a role in the pathogenesis of preeclampsia. Unfortunately, it is unknown whether there is an increase in intermediate monocyte before the onset of clinical signs of preeclampsia.

3.2. How is monocyte activation and maturation induced in preeclampsia?

It seems likely that the placenta plays a major role in monocyte activation and maturation during preeclampsia by direct contact or by producing factors, which may further activate and mature monocytes (see Fig. 2b). During the second stage of preeclampsia, the diseased placenta is thought to produce pro-inflammatory factors. In the peripheral circulation, many factors, which are able to affect monocytes, were increased in preeclamptic vs healthy pregnant women. Such factors are for instance anti-angiogenic factors (Maynard et al., 2003; Levine et al., 2004; Steinberg et al., 2009), placental microparticles (Redman and Sargent, 2000; Gohner et al., 2015) or ATP (Spaans et al., 2014d). The preeclamptic placenta has also been shown to produce various pro-inflammatory cytokines, such as TNF α , IL-1 β , IL-18 (Wang and Walsh, 1996; Benyo et al., 2001; Pang and Xing, 2003), with decreased production of anti-inflammatory cytokines such as IL-10 (Hennessy et al., 1999; Rein et al., 2003). These cytokines may also be involved in activating monocytes. Since monocytes themselves are also potent producers of cytokines, the activation of monocyte by placental factors and cytokines may in turn result in a vicious circle of monocytes activation and cytokine production leading to persistent increased monocyte activation, as well as activation of other immune cells, such as granulocytes, NK cells and endothelial cells in preeclampsia (Fig. 2b).

3.3. Monocytes in animal models for preeclampsia

As indicated above, due to the similarity in the fetal-maternal contact in the placenta, rodents are useful models to study immune responses in pregnancy. The advantage of the using the pregnant rat above the pregnant mouse is the fact that the rat not only has a hemochorial placenta, but the rat, and not the mouse, also shows deep trophoblast invasion into the uterine wall (Vercruysse et al., 2006; Soares et al., 2012; Spaans et al., 2014c). In accordance with the suggestion that an activated inflammatory response is part of the pathogenesis of preeclampsia, it has been shown in rat that activation of monocytes during pregnancy, by for instance LPS, ATP or TNF α , induced preeclampsia-like signs (Faas et al., 1994; LaMarca et al., 2008; Faas et al., 2010a). Such preeclampsia-

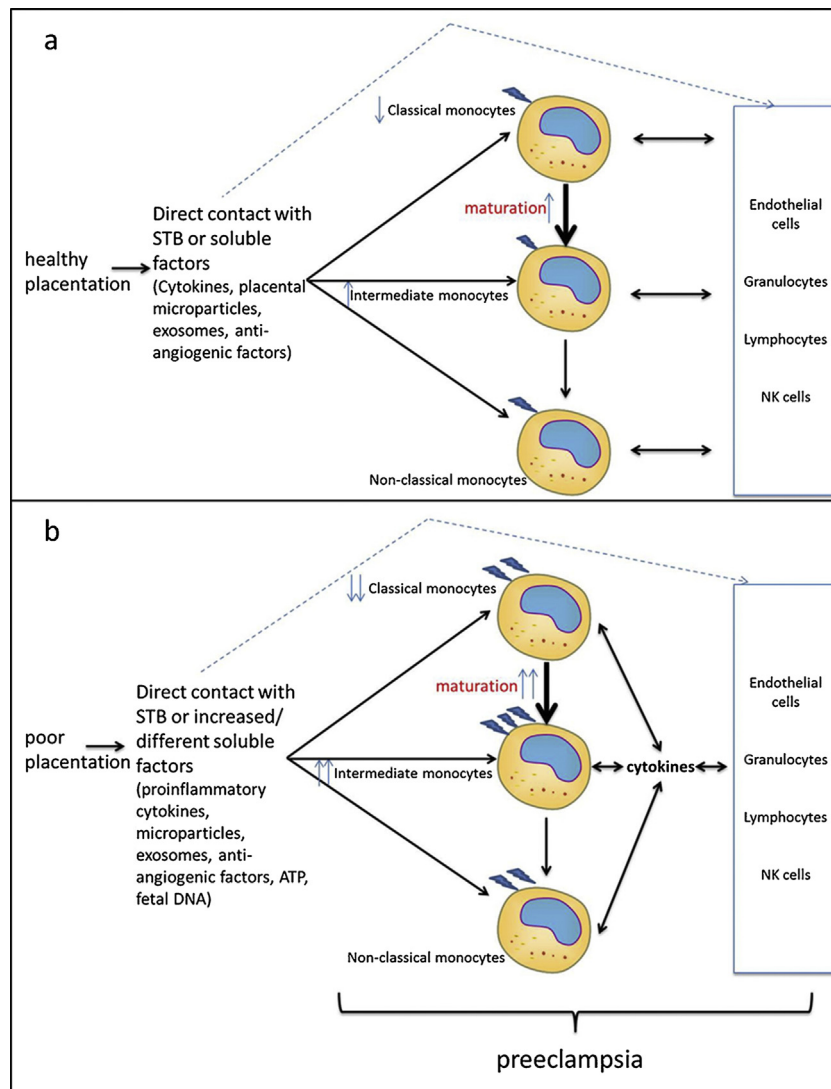


Fig. 2. Schematic overview of the (patho) physiological role of monocyte subsets during healthy pregnancy (a) and pre-eclampsia (b). During healthy pregnancy (a), the placenta, by direct contact and by producing soluble factors, activates monocyte subsets and induces increased maturation of classical monocytes toward intermediate monocytes. These monocytes interact with other immune cells and endothelial cells. During pre-eclampsia (2b), more and other soluble factors are produced from the diseased placenta, resulting in further activation of monocyte subsets and further maturation of classical monocytes toward intermediate monocytes, resulting in an increased number of intermediate monocytes. Activated monocytes produce various (proinflammatory) cytokines. These cytokines further activate the monocytes as well as other immune cells and endothelial cells. This vicious circle of activation of monocytes, other immune cells and endothelial cells finally results in the symptoms of pre-eclampsia, such as hypertension and proteinuria.

Adapted from (Faas et al., 2014b).

like signs were not induced in identically treated non-pregnant rats (Faas et al., 1994, 2010a). Infusion of LPS or ATP induced an inflammatory response, which was characterized by persistent general (Faas et al., 2000, 2004; Spaans et al., 2014b) and glomerular (Faas et al., 1995; Spaans et al., 2014b) inflammation. In other models for preeclampsia monocyte function per se has not been studied, however, in for instance the reduced uterine perfusion model, inflammation seems to play a role, since $\text{TNF}\alpha$ is increased in this model (Murphy et al., 2013). Also in the rat models of preeclampsia, monocyte maturation was induced: in the ATP model we observed increased numbers of non-classical monocytes and decreased numbers of classical monocytes in preeclamptic vs healthy pregnant rats (Melgert et al., 2012). In the LPS model (Faas et al., 1994), we found similar results (to be published). Together, these animal studies support the hypothesis that activation of monocytes in pregnancy may

result in preeclampsia-like signs, such as hypertension and proteinuria.

4. Conclusion

Healthy pregnancy is associated with activation and maturation of monocytes. We hypothesize that this is induced by direct or indirect contact of monocytes with the syncytiotrophoblast of the placenta. These factors, but also monocytes themselves, influence other immune cells and endothelial cells shaping the immune response during pregnancy (Fig. 2a). During preeclampsia, poor placentation results in production of more and different factors from the diseased placenta. This may induce further activation and maturation of the monocytes, but may also influence the other immune cells and endothelial cells. Moreover, activated monocytes produce cytokines, further activating the monocytes and other cells. This

results in a generalized inflammatory response, characteristic of preeclampsia and finally resulting in hypertension and proteinuria (Fig. 2b). Although at first sight, it seems dangerous to have a pro-inflammatory situation in healthy pregnancy, activation of these cells during pregnancy seems to play a physiological role in pregnancy. It has been suggested that pregnancy-induced monocyte activation is needed in order to compensate for the changes in the adaptive immune responses during pregnancy (Veenstra Van Nieuwenhoven et al., 2003b) and that monocyte activation may be needed to protect the immunological integrity of the mother.

It remains unknown why monocyte maturation occurs during pregnancy. It may be part of the pro-inflammatory response of pregnancy, since the intermediate subset has been shown to be increased in various inflammatory diseases. However, this subset of monocytes has recently been shown to have an immunoregulatory role, since this subset is less capable of activating and proliferating helper T cells and inducing IFN γ production by these helper T cells as compared with the other monocyte subsets (Liu et al., 2015). This subset also produces more IL-10, and induces more IL-10 in Treg cells as compared with the other monocyte subsets (Liu et al., 2015). This may suggest that the intermediate monocytes may have an important immunoregulatory role in healthy pregnancy. Their further increase in preeclampsia may be a compensatory mechanism in order to try and downregulate the preeclampsia-induced inflammatory response. It is unknown what drives monocyte maturation in pregnancy and preeclampsia. We have shown that one of the factors inducing monocyte maturation may be placental microparticles and exosomes (Gohner et al., 2015), suggesting that the placenta is involved in monocyte maturation in pregnancy.

It is also unknown whether and how the increased monocyte maturation and activation during pregnancy might be important for macrophage numbers in the decidua. Decidual macrophages are important for placental development. Although macrophages in many tissues may not arise from blood monocytes, but from the yolk sac, this appears to be different for mucosal tissues, such as the decidua, in which most macrophages seemed to be mainly derived from blood monocytes (Italiani and Boraschi, 2014). There is considerable debate on the fate of the different monocyte subsets (Italiani and Boraschi, 2014). It is, therefore, relatively unknown whether decidual macrophages arise from classical or non-classical monocytes during pregnancy. One paper, however, has shown that in mice mainly classical monocytes and not non-classical monocytes, infiltrate into the decidua in early pregnancy (Tagliani et al., 2011). Whether this is also true for late pregnancy remains to be established. Recent studies have shown the presence myeloid derived suppression cells (MDSC) in the decidua (Bartmann et al., 2016). Whether these cells arise from the blood monocyte population (classical or non-classical) or more directly from a monocyte precursor is subject of debate.

We suggest that further in vivo and in vitro studies should focus on the potential immunoregulatory role of the intermediate subset of monocytes and on monocyte maturation during pregnancy and preeclampsia. This should include studies into the fate of the monocytes, i.e. whether and how they are important for determining the macrophage population in the decidua. For studies like this, animal models are indispensable, since they allow us to mechanistically study the role of this subset, for instance by inducing inflammation, deleting or labeling a specific subset of monocytes.

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